Association of temporomandibular dysfunction with the 102T-C polymorphism in the serotonin receptor gene in Brazilian patients

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Abstract

Introduction: Serotonin is a key neurotransmitter in the central nervous system. It has been suggested that serotoninergic dysfunction mediates the pathophysiology of temporomandibular dysfunction (TMD). Polymorphisms in the serotonin receptor gene (*HTR2A*) can alter its transcription, affecting the number of receptors in the serotoninergic system, altering nociceptive pain and hyperalgesia in TMD. The aim of this study is to investigate the association of the *102T-C* polymorphism in the *HTR2A* gene in Brazilian patients with TMD.
Material and methods: This cross-sectional study examined 100 patients, of both genders, with TMD as index cases and 100 healthy volunteers as controls, also of both genders. DNA was extracted from peripheral blood leukocytes, and the site that encompassed the polymorphism in the *HTR2A* gene was amplified by polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP).
Results: Our results revealed that there were significantly more females among index cases compared with the control group ($p < 0.05$). The *CC* genotype of the *102T-C* polymorphism was more frequent in patients with TMD vs. controls (OR: 2.25; 95% CI: 1.13–4.46; $p < 0.05$).
Conclusions: The present study supports the view that the 102T-C polymorphism in the *HTR2A* gene is associated with TMD in this studied Brazilian population.

Key words: myofascial pain, temporomandibular joint, *HTR2A* gene, molecular analysis.

Introduction

Of the disorders that cause orofacial pain, the most common are disorders in the temporomandibular joint. Most patients with temporomandibular dysfunction (TMD) develop local joint pain, primarily affecting the muscles of mastication [1].

Temporomandibular dysfunction-related facial pain has been reported in 4% to 12% of the population, wherein women experience a greater frequency and severity of TMD compared with men [2, 3]. The signs and symptoms of TMD are more common in patients aged 20 to 40 years [3]. It rarely progresses into severe or chronic pain (if it persists for over

6 months) and may be associated with psychological disorders [4, 5].

Recently, genetic factors have been implicated in the development of pain in masticatory muscles, causing myofascial pain syndrome of the stomatognathic system-particularly those that are related to polymorphisms in the serotonin receptor gene (*HTR2A*) [6–8]. Serotonin (5-hydroxytryptamine – 5-HT), a neurotransmitter in the central nervous system, regulates several physiological and visceral functions. Thus, abnormalities in the serotoninergic system underlie many human illnesses, such as depression, headache, epilepsy, obsessive-compulsive disorder, affective disorders, sleep apnea, and TMD [7–10].

5-Hydroxytryptamine is considered the most important neurotransmitter that regulates endogenous mechanisms of pain [11, 12]. A peripheral mechanism has been proposed to be the cause of chronic muscle pain, because mechanical overload and the consequent muscle hypoxia and microcirculation disorders have been implicated as causes of chronic myalgia due to the sensitization and activation of muscle nociceptors by algogenic substances [13, 14].

These findings suggest that 5-HT in deep craniofacial tissue induces peripheral sensitization, leading to the development of hyperalgesic nociceptive responses, and mediates the development and maintenance of chronic orofacial pain in TMD [7, 8]. Thus, this neurotransmitter modulates nociceptive pain and hyperalgesia using central and peripheral mechanisms [7, 8, 15–17].

Like other neurotransmitters, serotonin is released into the synaptic cleft and exerts its effects on specific receptors on the postsynaptic membrane. 5-Hydroxytryptamine acts through a large family of 5-HT receptors, of which the 5-HT2 receptors comprise 3 subtypes – the 5-HT2A, 5-HT2B, and 5-HT2C receptors, which are similar in molecular structure, pharmacology, and signaling [18, 19]. Some receptor subtypes in central and peripheral nervous tissue modulate pain. The 5-HT2A receptors in postsynaptic terminals mediate serotonin-induced hyperalgesia and are associated with the potentiation of inflammatory pain in various diseases that are caused by dysfunction of the serotoninergic system, particularly TMD [7, 8].

Polymorphisms in the *HTR2A* gene are associated with many diseases, including TMD, that affect the serotoninergic system. Thus, changes in serotoninergic neurotransmitters can lead to alterations in muscular tone. Recently, a silent polymorphism in the *HTR2A* gene was identified, defined by a *T*-to-*C* transition at position 102 in exon 1, in 36.5% of patients with TMD [6].

The 102T-C polymorphism might be a functional polymorphism, because the *T* and *C* alleles determine gene expression – the *C* allele, compared with the *T* allele, decreases gene expression and, consequently, the amount of 5-HT2A receptors [20]. On declines in the amount of postsynaptic receptors, the concentration of serotonin in the synaptic cleft increases; thus, the resulting increase in serotonin reuptake into the presynaptic terminal reduces postsynaptic serotonin levels. These low serotonin levels are insufficient to stimulate the central descending analgesic system, triggering sensations of pain or hyperalgesia in TMD [6–8].

This study aimed to investigate the association of the *102T-C* polymorphism in the *HTR2A* gene in Brazilian patients with temporomandibular dysfunction.

Material and methods

We studied 100 index cases with TMD (44 males and 56 females, age range 17 to 76 years) who were admitted consecutively to the Department of Otolaryngology and Head and Neck Surgery, Faculty of Medicine of São José do Rio Preto, São Paulo, Brazil (FAMERP), between May 2009 and December 2010. The diagnosis of TMD was based on a questionnaire and clinical investigation, using data from the Table Procedures Clinical Investigation for TMD. Both methods are in accordance with the American Academy of Orofacial Pain [21].

For case selection, the following criteria were considered: *Inclusion criteria:* 1) Presence of muscle or joint pain for more than 30 days and not exceeding 6 months. 2) Presence of noises (clicks) in the temporomandibular joint. 3) Asymmetrical mandibular movements. All the patients had to show all three inclusion criteria simultaneously. *Exclusion criteria:* Patients with somatic, rheumatological, neurological, or psychiatric illnesses, mental retardation, alcoholism or drug dependence were excluded.

The 100 control subjects (78 males and 22 females, age range 18 to 63 years) were included based on the following simultaneous criteria: 1) no joint or muscle pain, 2) absence of noises (clicks) in the temporomandibular joint, and 3) symmetrical mandibular movements. Similarly, patients with mental retardation, drug or alcohol dependence, or somatic, rheumatological, neurological, or psychiatric illnesses were excluded.

The patients and control subjects were Caucasian, from the same geographic area – São Paulo State, (Brazil) – and grouped by age: adolescents (11 to 17 years), young adults (18 to 40 years), adults (41 to 65 years), and elderly (> 65 years) [22].

This study was approved by the Research Ethics Committee (FAMERP) (#393/2008).

Clinical investigation

For the clinical investigation, 4 basic procedures were performed to diagnose TMD: measurements

of the extent and symmetry of mandibular movements; examination of joint palpation and facial muscles to identify the anatomical origin of the pain; auscultation of the joint during functional movements to determine the presence of joint noise; and analysis of occlusion [21].

Molecular analysis

Genomic DNA was isolated from peripheral blood leukocytes using the Illustra Blood Genomic Prep Mini Spin Kit (GE Healthcare UK Limited™, UK) as per the manufacturer's protocol. To detect the *102T-C* (GenBank NM_000621.3; rs#6313) polymorphism, nuclear DNA fragments that encompassed the polymorphic site in the *HTR2A* gene (MIM ID *182135) were amplified by PCR and digested with 10 U MspI (New England Biolab®) for 2.5 h at 37°C [6].

Polymerase chain reation conditions

Standard polymerase chain reaction (PCR) was performed in 25 µl, containing 200 ng genomic DNA, 10 pmol of each primer, and FideliTaq™ PCR Master Mix (2x) (GE Healthcare UK Limited™, UK), as per the manufacturer's protocol. Published primer sequences were used to analyze the *102T-C* polymorphism [6], and PCR was performed as follows: initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 2 min, followed by a final extension for 10 min at 72°C.

The products from the PCR reaction were analyzed by electrophoresis on a 2% agarose gel in 1X TBE buffer. The *102T* allele was represented by an uncut 342-bp PCR product, and the *102C* allele consisted of 2 fragments of 217 bp and 125 bp.

To avoid bias in the molecular analysis and final results, all DNA samples were analyzed without knowledge of the patients' clinical characteristics.

Statistical analysis

Statistical analyses were performed using Graph Pad InStat, version 3.00 (Graph Pad Software Inc, San Diego, California, USA). Qualitative variables were expressed as a percentage (%). Continuous variables were expressed as a mean (M) and standard deviation (SD), and because the mean age was not normally distributed between both groups, it was compared using the Mann-Whitney test. Correlations between variables were assessed using the χ^2 test and odds ratios with a confidence interval of 95% (95% CI) at a significance level of 5%. All samples were tested for Hardy-Weinberg equilibrium.

Results

Of the 100 index cases, 44 (44%) were male and 56 (56%) were female. Males ranged in age from 17 to 76 years (mean: 37.9 years; SD ±12.8), and females ranged in age from 18 to 68 years (mean: 34.4 years; SD ±12.8).

Of the 100 control patients, 78 (78%) were male and 22 (22%) were female. Control males ranged in age from 18 to 63 years (mean: 35.4 years; SD ±11.6), and control females were aged 19 to 54 years (mean: 32.5 years; SD ±11.6).

With regard to the demographics (Table I), there were more females (56%) in the index case group and more males in the control group (78%) ($p < 0.0001$). In both groups, the percentages of those in the young adult and adult age brackets combined were highest – 97% and 100%, respectively – but this difference was not significant compared with the other age groups ($p > 0.05$). The relationship between mean age of both groups was not significant ($p > 0.05$).

Regarding the genotype frequencies, the *TT* genotype (wild-type) was more prevalent in the control group (37%), and the *CC* genotype (polymorphic) was more common in the index cases (30%) ($p = 0.015$) (Table II).

There were more females (44%) in the index group and more males (67%) in the control group with the *TT/TC* genotypes, and 18% and 11% of males, respectively, harbored the *CC* genotype. The relationship between gender and genotype was significant in both groups ($p = 0.0015$) (Table III).

Table I. Demographics of index cases ($n = 100$) and the control group ($n = 100$)

Variables	Index cases	Controls	Value of *p*
Gender	*n* (%)	*n* (%)	< 0.0001*
Male	44 (44)	78 (78)	
Female	56 (56)	22 (22)	
Age range	*n* (%)	*n* (%)	0.9080*
Adolescent	1 (1)	0 (0)	
Young adult	65 (65)	69 (69)	
Adult	32 (32)	31 (31)	
Elderly	2 (2)	0 (0)	
Age [years]	M ± SD	M ± SD	0.5808†
	35.9 ±12.9	34.7 ±11.6	

χ^2 test, †Mann-Whitney test, M ± SD – mean ± standard deviation

Table II. Distribution of genotypes of the *102T-C* polymorphism in the *HTR2A* gene in index cases and controls

Genotype	Index cases *n* (%)	Controls *n* (%)	Value of *p**
TT	16 (16)	37 (37)	0.0015
TC	54 (54)	47 (47)	
CC	30 (30)	16 (16)	

χ^2 test

Table III. Distribution of genders in index cases and control group with regard to the *102T-C* polymorphism in the *5-HTR2A* gene

Genotype	Index cases		Controls		Value of *p**
	Male, *n* (%)	Female, *n* (%)	Male, *n* (%)	Female, *n* (%)	
TT	7 (7)	9 (9)	27 (27)	10 (10)	0.0028
TC	20 (20)	35 (35)	40 (40)	7 (07)	
CC	17 (17)	12 (12)	11 (11)	5 (5)	

χ^2 test

Table IV. Genotypic relationship between index cases and control group for the *102T-C* polymorphism in the *HTR2A* gene

Genotype (Index cases × controls)	Odds ratio (OR)	95% CI	χ^{2*}	Value of *p**
TT (16 × 37)	0.32	0.16–0.63	10.27	0.0014
TC (54 × 47)	1.32	0.76–2.31	0.72	0.3961
CC (30 × 16)	2.25	1.13–4.46	4.77	0.0289

*95% CI – 95% confidence interval, *χ^2 test*

Table V. Allelic relationship between index cases and control group for the *102T-C* polymorphism in the *HTR2A* gene

Alleles (Index cases/controls)	Odds ratio (OR)	95% CI	χ^{2*}	Value of *p**
T (86/121)	0.49	0.33–0.73	11.57	0.0007
C (114/79)	2.03	1.36–3.02		

*95% CI – 95% confidence interval, *χ^2 test*

The index cases had a significantly lower frequency of the *TT* genotype compared with the control group ($p = 0.014$). However, the index cases had the *CC* genotype with a higher frequency when compared with controls ($p = 0.0289$). There was no significant difference in the frequency of the *TC* genotype between groups ($p > 0.05$) (Table IV).

With regard to allele frequencies, the index cases had a significantly lower frequency of the *T* allele (wild-type) and a higher frequency of the *C* allele (polymorphic) compared with controls ($p = 0.0007$) (Table V).

The allelic frequencies for the index cases were $T = 0.43$ and $C = 0.57$ and $T = 0.60$ and $C = 0.40$ for the controls. Both groups were tested for Hardy-Weinberg equilibrium, bearing no significant differences ($p > 0.05$) between the observed and expected genotypic values for the *102T-C* polymorphism (index cases $\chi^2 = 1.03$, $df = 1$, $p = 0.31$; controls $\chi^2 = 0.03$, $df = 1$, $p = 0.87$).

Discussion

The molecular basis of temporomandibular dysfunction (TMD) is still unknown and merits further research. Genetic factors have been implicated in the development of pain in masticatory muscles, causing myofascial pain of the stomatognathic system, especially the factors that are associated with polymorphisms in the serotonin receptor gene (*HTR2A*) [7, 8].

The 5-HT2A receptor is an essential component of the serotoninergic system, and its expression is under genetic control. Thus, polymorphisms in genes encoding the receptor can affect its function and thus modulate serotoninergic activity, causing changes in muscle tone [18].

This study evaluated the prevalence of the *102T-C* polymorphism in the *HTR2A* gene in patients with and without TMD, because there have been no Brazilian studies on this topic; only one study in the international literature has reported the frequency of this polymorphism and its association with TMD [6], highlighting the significance of this study.

To assess the results of genetic association studies, the test and control groups should have the same ethnic and geographical origins, because of the genetic basis of diseases, such as polymorphic configurations, which can vary between regions and populations. The influence of ethnic and geographic diversity can be extensive and should be considered in genetic studies [6, 22–26]. In this study, despite the significant miscegenation in Brazil, only Caucasian patients and controls were included, all of whom were from the same geographical area.

It is widely accepted that TMD is more prevalent in females, affecting those aged 20 to 40 years [3]. Epidemiological studies of the Brazilian population observed a high prevalence of females among patients with TMD, the majority of whom were in the age range 21–30 years [27, 28]. Because women tend to seek treatment for their TMD more frequently than men, attempts have been made to explain these differences in terms of behavioral, psychosocial, hormonal, and constitutional disparities, although no conclusive results have been generated [3, 5].

In this study, based on the literature [3, 5, 27, 28], there was a predominance of females in the TMD group, outnumbering men 1.2 : 1; but in the control group, males predominated by 3.5 : 1. The average age of females in the TMD group (34.4 years) and control group (32.5 years) was slightly higher than what has been described in the Brazilian literature [27, 28] but is within the range of the international literature [3, 5].

In an international study that associated the *102T-C* polymorphism in the *HTR2A* gene to TMD, 63 patients and 54 controls of both genders were analyzed; the *CC* genotype (polymorphic) was expressed in 36.5% of patients with TMD, and the *TT* genotype (wild-type) was noted in 27.8% of controls [6].

Similarly, we compared the genotypic and allelic frequencies between patient and control groups. The *CC* genotype (polymorphic) was more prevalent among index cases (30%), and the *TT* genotype (wild-type) predominated in the control group (37%). Consequently, the C allele was more frequent in the TMD group, and the T allele was more frequent in the control group.

Recent findings [1, 6–8] indicate that the variant genotypes of *HTR2A* gene, which have disparate levels of activity, could affect or modify serotoninergic activity by reducing the number of postsynaptic receptors, resulting in a lower concentration of serotonin in the extracellular space – particularly active serotonin [1, 6–8].

Low serotonin levels are insufficient to stimulate the central descending analgesic system, triggering the feeling of pain or hyperalgesia in the TMD. Thus, these genotypic variants may have different molecular functions in TMD; alternatively, they might mediate its pathogenesis or determine a genetic predisposition to it, although TMD does not seem to have familial patterns of pathology [1, 6–8].

Like many other functional pain syndromes, however, the underlying mechanisms that contribute to craniofacial pain in TMD remain poorly understood. This lack of understanding is compounded by the often poor correlation between the severity of TMD-related complaints of pain and definitive pathophysiological evidence, such as tissue damage and degeneration, in painful tissues. Thus, in some patients with TMD, pain has been proposed to result from altered processing of pain by the central nervous system, which is attributable to specific heritable genes, such as *HTR2A* gene [1].

Despite the predominance of females in the TMD group and males in the control group in this study, there was a higher percentage of males with the *CC* genotype in both groups – 17% and 11%, respectively. In the reference study [6], despite the predominance of males in both groups, there was no mention of the predominant gender for the polymorphic genotype (*CC*); thus, it was not possible to determine whether these gender differences explain the findings in our study with regard to the genotype distribution.

Serotonin and its receptors have been the focus of many reports and reviews, but only one has examined their interaction with TMD [6]. Other genetic studies should be performed to determine the complexities of TMD and develop new approaches for this dysfunction, which can result from the interaction between multiple genes and environmental factors. Such knowledge can increase our understanding of the physiology and pathophysiology of myofascial pain and facilitate the discovery of the molecular causes of TMD, providing new perspectives on early diagnosis and pharmacological therapies and stimulating research to determine how the serotoninergic system influences the modulation of pain [1]. The genetic association with TMD strengthens the serotoninergic system dysfunction hypothesis with regard to TMD pathogenesis [1, 6].

The potential limitations of these data merit consideration, because despite our positive findings, they should be interpreted with caution and must be corroborated by independent and multicenter studies to determine the real prevalence of the *102T-C* polymorphism in the *HTR2A* gene and its association with TMD in the Brazilian population. Therefore, it is necessary to validate our data in prospective studies with a larger population size. Second, as our study was conducted in a sample of Brazilian patients, extrapolation of the data to other ethnic groups should be done with great caution.

In conclusion, the present findings suggest that there is evidence of an association between the *102T-C* polymorphism in the *HTR2A* gene and TMD in this studied Brazilian population.

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